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THE FASEB JOURNAL • REVIEW • www.fasebj.org

Recommended housing densities for research mice: filling the gap in data-driven alternatives

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ABSTRACT: Space recommendations for mice made in the *Guide for Care and Use of Laboratory Animals* have not changed since 1972, despite important improvements in husbandry and caging practices. The 1996 version of the *Guide* put forth a challenge to investigators to produce new data evaluating the effects of space allocation on the well-being of mice. In this review, we summarize many studies published in response to this challenge. We distinguish between studies using ventilated or nonventilated caging systems and those evaluating reproductive performance or general well-being of adult mice. We discuss how these studies might affect current housing density considerations in both production and research settings and consider gaps in mouse housing density research. Additionally, we discuss reliable methods used to monitor and quantify general well-being of research mice. Collectively, this large body of new data suggests that husbandry practices dictating optimal breeding schemes and space allocation per mouse can be reconsidered. Specifically, these data demonstrate that prewean culling of litters has no benefit, trio breeding is an effective production strategy without adversely affecting pup survival and well-being, and housing of adult mice at densities of up to twice current *Guide* recommendations does not compromise well-being for most strains.—Svenson, K. L., Paigen, B. Recommended housing densities for research mice: filling the gap in data-driven alternatives. FASEB J. 33, 3097–3111 (2019). www.fasebj.org

KEY WORDS: well-being · husbandry · physiology

Husbandry practices and other environmental conditions, under which mouse models of human disease are developed and used, vary widely among research institutes. These variables affect behavior and ultimately well-being of research mice and collectively create an obstacle to reproducibility and translation of research results. Unchecked, this aspect of experimental design can induce chronic psychosocial stress and associated downstream changes in basic physiology and behavior that may confound interpretation of results. An understanding that mice are naturally social animals is of fundamental importance when using them in the research setting. What are optimal housing conditions? Do they vary by strain? Can a "one size fits all" recommendation be determined to minimize effects of isolation or crowding?

Housing density standards for research mice set in 1963 in the first edition of the *Guide for the Care and Use of Laboratory Animals (Guide)* (National Institutes of Health, Bethesda, MD, USA) were modified in the second edition, published in 1972. However, space recommendations per mouse, based on their weight, have not changed in subsequent editions, published in 1974, 1978, 1980, 1985, 1996, and 2011, despite improvements in husbandry and caging practices. Currently, the space recommendations are 6 in^2 for mice <10 g, 8 in^2 for mice up to 15 g, 12 in^2 for mice up to 25 g, and >15 in^2 for mice weighing more than 25 g (1).

The seventh edition of the *Guide*, published in 1996, recognized a paucity of information for supporting these space recommendations and encouraged the use of alternatives as long as they were data-driven and based on sound science (2). With a 14-yr gap before publishing the eighth edition in 2011, the Committee for the Update of the *Guide* noted that "important new research findings might wait more than a decade before being reflected in recommended practice" (1). Much of the relevant data from newer studies addressing space recommendations was recently reviewed by Whittaker and colleagues (3). Since that review several additional important studies have been published that include a broad range of widely used mouse strains. Moreover, the increasing replacement of static caging systems with individually ventilated cages (IVC) necessitates an evaluation of caging practices in the two systems. Ventilated systems both improved cage air quality for mice (4) and reduced exposure of personnel to mouse allergens (5). Frequency of cage changing may also be reduced by using IVC systems (6).

ABBREVIATION: IVC, individually ventilated cage

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Here, we summarize conclusions from studies of housing density using ventilated and nonventilated cages and discuss gaps yet unfilled in this area of animal husbandry research. We highlight studies aimed at determining the impact of housing density on breeding and reproduction (including culling of litters) as well as on baseline physiology, behavior, and well-being of nonbreeding mice used in research. Careful consideration of home cage conditions that may impact these parameters is an important part of a well-designed experimental plan. Limiting the number of mice housed within a specified area implies that a threshold exists above which animal well-being is compromised. This conclusion was not previously supported by data but rather by best judgment of professionals. However, considerable data now exist from studies of densities exceeding *Guide* recommendations. Many of these studies, summarized in this review, were conducted in direct response to the recognition in the seventh edition of the *Guide* that evidence to support changing recommendations is lacking.

One of the most widely used mouse strains in biomedical research is C57BL/6 (B6), generally acquired from 1 of 3 vendors: J, The Jackson Laboratory (Bar Harbor, ME, USA); Tac, Taconic; Crl, Charles River Laboratories (Wilmington, MA, USA). Therefore, it is not surprising that B6 is commonly used to evaluate effects of housing density. Recent studies, however, include other commonly used strains and reveal strain-specific differences in response to cage density. The use of multiple cage types and adequate numbers of replicate experimental groups improves the reliability and applicability of these studies. Commonly used mouse cage types include a "duplex" box with two small separate pens, each with 51.7 in^2 floor space, a medium sized "shoebox" cage with 67.6 or 78.1 in^2 of floor space, and larger "weaning" cages with 112.9 in^2 of floor space. Some studies used a fixed number of mice housed in differently sized cages to evaluate the effects of density on a fixed social group. Others used variable numbers of mice housed in a fixed cage size, which varies both housing density and the size of the social group.

To organize these studies, we have separated them into 4 groups: Reproduction studies using IVC (IA) or static caging (IB) and studies using nonbreeding cohorts in either IVC (IIA) or static caging (IIB). Gaps to consider filling in future density studies are identified. We begin by summarizing current methods commonly used to evaluate the well-being of research mice.

ASSESSING WELL-BEING IN LABORATORY MICE

With increasing interest in more fully characterizing mouse models, many institutions have established core facilities with specialized testing and phenotyping capabilities. In the absence of such resources, many of the measurements described in this review may be obtained without specialized equipment and will serve to describe and test well-being. Standard operating procedures for similar assays often vary among research laboratories, and small differences in procedure can have important effects on outcome (7). Investigators can readily access protocols for many commonly used assays for behavior, cardiovascular, metabolic, sensory, and other physiologic measurements from open access sources such as the International Mouse Phenotyping Consortium [*http://www.mousephenotype.org/* (8)]. Efforts to harmonize methods for testing well-being in mice will allow valid comparisons to be made across studies and facilitate reproducibility in future experiments.

Comprehensive high-throughput phenotyping is now widely used to more fully characterize inbred and engineered mouse strains (9–12). These range from broad based, noninvasive analyses to highly specialized and sophisticated testing platforms. In cases where density studies are planned with limited resources, there are many parameters that can be easily measured without specialized equipment. Commonly used measures from studies included in this review are provided in **Table 1** and briefly discussed below. A fundamental first step in any mouse experiment is to ensure that inherent phenotypes of some strains (occurring at birth or with development) such as deafness [*e.g.*, A/J and DBA/2J (13)] or visual impairment [*e.g.*, C3H/HeJ and BALB/cByJ (14)] do not confound results.

Behavior (aggression, anxiety, distress)

Behavior encompasses an expansive repertoire of parameters used to describe psychiatric and psychologic profiles, many of which can be modified by environmental changes and lead to social stress. Psychosocial stressors are implicated in the development of anxiety and mood disorders (15), and the development of chronic stress can be measured in a variety of simple to complex systems. Sampling can be from plasma, urine, or feces to measure stress hormone levels (*e.g.*, corticosterone, testosterone) or may involve more complex continuous behavioral monitoring. Aggression, often accompanied by fighting, is often monitored by regular observation of the appearance of bites or scratch wounds among cage mates. Manual interpretation of video footage has been widely used to score aggressive behaviors (16, 17), but analysis of such video recordings is moving to machine-learning and other automated approaches for interpretation and quantification (18, 19). A comprehensive discussion of measurement and management of aggression in group-housed laboratory mice has been discussed in a recent perspective (20).

Many noninvasive strategies are available for measuring mouse behaviors. The open field arena is used to evaluate anxiety-related behavior by observing where a mouse prefers to spend time in an arena, either in the center (less anxious) or in the periphery (more anxious). Activity and exploration are also measured in this test. The light/dark box and elevated plus maze tests measure similar behaviors. The tail suspension test quantifies behavioral despair and is used to assess chronic or induced depression in mouse models (21).

Behavioral testing typically involves moving the test mouse from the home cage to the measurement apparatus, recording data, and returning the animal to its home cage, a process that may influence intrinsic behavior. Recent technologies have emerged using video monitoring and sophisticated data acquisition and analysis algorithms (22–24) to observe continuous behavior of multiple animals within their home cages. These systems may allow

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TABLE 1. *Commonly used measures of well-being for laboratory mice*

Biologic process	Test methodology	Parameters tested	Rationale
Behavior: aggression, anxiety, distress	Open field arena	Time spent in center *vs.* periphery	Exploration; may reveal anxiety. Anxious mice are thought to spend less time in center
	Light/dark box	Time spent and mobility in light or dark side of a box	Exploration; may reveal anxiety. Anxious mice are thought to spend less time in light side
	Tail suspension test	Time to immobility	Measures chronic or induced despair/depression; longer time to immobility suggests more despair
	Elevated plus maze	Time spent in open or closed arms	Measures anxiety; more time spent in closed arms suggest anxiety
	Home cage monitoring: automated or observational	Fighting, bite or scratch wounds, social behaviors	Increased aggression may be due to chronic social stress
	Hormone measurements	Corticosterone, testosterone	Stress is indicated by increased production of stress hormones
	Organ weight	Adrenal glands, testes	Increased organ weight due to increased production of stress hormones
Immune function	Organ weight	Spleen, thymus	Increased weight due to increased production of immunologic factors or stress hormones
	Flow cytometry	T-cell subpopulations	Immunologic status may change with chronic stress
	Hematology	Complete blood counts	General well-being
Cardiovascular	Electrocardiogram, blood pressure, telemetry	Heart rate	Increases with increasing stress
	Organ weight	Heart weight	May increase with increased chronic stress
Reproduction	Breeding success	Litter size, time between litters, number of litters	Small litters and increased time between litters may indicate distress
	Litter success	Pup weight, survival, play behavior, press posture, mortality	Viability of pups reflects behavioral environment
General health	Growth	Body weight increase with development	Abnormal weight patterns may reflect social stress
	Body composition (e.g., DEXA)	Fat tissue mass, bone mineral density	Chronic stress mice may inhibit body fat accumulation and reduce bone density
	Food and water consumption	Daily or weekly intake	Abnormal eating or drinking patterns may reflect social stress
	Mortality	Number and timing of deaths	Failure to thrive may reflect social stress
	Clinical chemistries	Plasma lipids, glucose	General well-being
	Cage microenvironment (air quality)	CO_2, NH_3, temperature, relative humidity; nasal pathology	Poor cage air quality compromises well-being; nasal pathology reflects deleterious effects of high concentrations of inhaled irritants

detection of more subtle changes in behaviors, but their use in density studies has not yet been reported.

Immune function

Chronic social stress has been associated with altered immune function (25–28). Spleen and thymus weight can serve as a surrogate for production of immune cells, with heavier weights reflecting increased stress. Flow cytometry analyzes specific cell populations and can be performed longitudinally using serial analysis of peripheral blood lymphocytes or terminally using spleen or thymus tissue. Spleen is more commonly used because T-cell populations may be unreliable if thymus involution has occurred.

Cardiovascular function

Heart rate, a primary indicator of chronic stress, can be measured in unanesthetized mice either with electrocardiogram or blood pressure equipment. Heart rate variability measures adaptation to stress stimulators and is often an additional useful output of these methods. Telemetry can provide similar data but requires surgery and recovery, which may confound results.

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Reproduction

Parameters used to describe reproductive success include litter size, time between litters, pup weight, and pup mortality. Often, second generation pups are also evaluated for reproductive indices to assess the impact of conditions in the cages from which they were weaned.

General health

Daily well-checking can be used as a measurable parameter in density studies by, for example, recording observations of aggression and mortality. Growth is followed with regular weighing of mice throughout a study. Plasma chemistry profiles are also useful for discerning general health. Differences in glucose, triglycerides, and total cholesterol have been reported in response to stressors (29, 30). Most diagnostic reagents developed for humans have been established for use in mouse serum or plasma. Organ weights are surrogates for production of stress-induced functional cells and products such as inflammation (spleen, thymus) and hormone production (adrenal) and kidney.

Cage microenvironment

Monitoring in-cage temperature, relative humidity, ammonia, and carbon dioxide levels requires suitable equipment. It is important that sensors are installed in such a manner to reliably reflect average levels within the cage while remaining out of reach of mice.

Testing multiple parameters

Measuring multiple parameters in a single study will facilitate interpretation of results, because simultaneous measures may independently support a conclusion. For instance, better well-being can be assessed from a combination of results such as reduced heart rate, increased body fat, and lower adrenal weight. Moreover, using appropriate statistical methods to analyze data and compare outcomes among experimental groups, including correction for multiple testing such as with the Bonferroni correction, is critical to interpretation of results, something that not all studies have done. When statistically significant differences are found between groups, their interpretation requires examining the biologic relevance of the difference, whether values remain within normal physiologic parameters and whether differences pose a risk to well-being.

IA. REPRODUCTION AND CULLING STUDIES: VENTILATED CAGING

The 1996 *Guide* gives no specific space recommendation for breeding dams and litters. To adhere to the recommendation of 6 in^2/mouse weighing <10 g (*i.e.*, pups), a standard 51.7 in^2 pen, for instance, would allow no more than 1 dam and a litter of 6 pups. Therefore, many institutions cull litters to a number of pups appropriate to cage size used. The rationale for this practice was based upon the premise that reducing the number of pups would improve nutrition and growth for the remaining pups. Breeding strategies also vary among laboratories and include paired (1 female and 1 male), trio (2 females and 1 male), and harem (3 females and 1 male) configurations. Some breeding schemes involve removal of the male upon the birth of pups. Two studies evaluating culling and 3 investigating reproductive success in ventilated caging are summarized in this section. Outbred (ICR, Swiss Webster), hybrid (B6129SF1), and inbred (B6, 129) were used in these studies.

O'Malley *et al.*

In O'Malley *et al.* (31), with use of 28 pregnant females of outbred ICR mice, whose average litter size was 11.4, litters were either culled to 6 nursing pups or left intact. Mice were provided with 65 in^2 of floor space. Fecal corticosterone and growth rates of pups showed no statistical differences between the groups for sex ratio, total number of pups weaned, growth rate, or fecal corticosterone. Reproductive performance (time to first delivery, litter size, growth rate of pups, percentage of pups weaned) of weaned pups did not differ between groups.

Based on these results, the authors concluded that 65 in^2 provides adequate floor space for a dam with as many as 13 pups and that culling litters has no measurable benefit to remaining pups. They also advocated that a dam with litter should be considered a single biologic unit, rather than counting individual pups toward the recommended space allocation.

Whitaker *et al.*

Using 190 trio breedings (2 females and 1 male) of strain C57BL/6Tac, Whitaker *et al.* (32) used cages that provided 82 or 124 in^2 of floor space, with and without enrichment in each cage type. Cage size had no effect on the number of pups born or weaned per litter, or on time between litters, but larger cages were associated with lower pup weights at 21 d of age. Cages with enrichment showed an increase in number of pups weaned and in weights of pups at 21 d of age, regardless of cage size.

DiVincenti *et al.*

The DiVincenti *et al.* (33) study compared cage microenvironment (NH_3, CO_2, relative humidity) in pairs and trios of continuously breeding Swiss Webster mice in cages with 75 in^2 of floor space. Fifteen cages per breeding configuration were used. Litter size was standardized to 6 pups per litter for each breeding scheme. Measures of breeding performance were not made in this study. Intracage temperature increased with time for both pair and trio breeding units, but never reached the metabolically stable thermoneutral zone of 30–33°C (34). Relative humidity did not differ between pairs and trios, regardless of number of pups in the cage. CO_2 concentrations stayed

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below 5000 ppm throughout the study for both configurations. Average ammonia levels did not differ significantly between breeding formats, but as expected, increased with second litters. For example, with trio breeding, ammonia levels rose to 900 ppm, significantly higher than in pair breeding, but this occurred after 14 d between cage changing. This can be avoided if trio configurations are changed weekly. Histopathology of upper airways, where effects of inhaled irritants are manifest, were evaluated in 10% of adult breeders and weanlings. Some increase in nasal lesions occurred with cage density, but other lesions arose in the upper respiratory tract. Investigators noted that no behavioral changes or signs of distress were observed in any of the mice in the study and that all mice appeared clinically healthy.

Although the authors concluded that cage microenvironment in both breeding configurations may be detrimental to animal health, the data do not support this conclusion, because no cages exceeded standards for microenvironment conditions and productivity and general good health and well-being were observed. More frequent cage changing or higher air exchange rates would alleviate increased ammonia levels, especially in trio breeding units.

Paigen *et al.*

The Paigen *et al.* (35) study evaluating 468 litters from 78 trios of a hybrid mouse strain (C57BL/6J × 129S1/SvImJ) reached similar conclusions about culling as the O'Malley study. In addition to evaluation of pup growth and survival, a subset of the culled and the intact litters was allowed to grow to 3 mo of age to evaluate whether culling had long-term effects on growth and well-being. Growth, health, and relevant physiologic parameters (mortality, organ weights, bone mineral density, percent body fat, select whole blood, and electrocardiogram parameters) did not differ between the 2 groups. The authors concluded that culling was not beneficial to remaining pups and may be an unnecessary step in animal husbandry practices.

Braden *et al.*

Braden *et al.* (36) examined maternal and weanling behavior of C57BL/6J and 129S6/SvEvTac in 14 pair, 10 trio, and 10 harem (3 females and 1 male) breeding configurations for each strain. Trio and harem breeding produced higher weanling weights in both strains. Although no negative effects on behavior or pathology were found in mice in harem breedings, ammonia levels were higher than recommended, and, therefore, these authors suggest avoiding this configuration. Strain B6 produced significantly fewer pups in the harem configuration. Harem breeding produced no welfare benefits over trio breeding. Based on their findings, the authors concluded that both pair and trio breeding in a standard shoebox cage (67 in^2) are appropriate and beneficial breeding strategies and that harem breeding produces no welfare benefits over trio breeding.

IB. REPRODUCTION STUDIES: STATIC CAGING

In static cages (nonventilated), 2 recent studies compared breeding performance between pair and trio configurations. A third study directly compared breeding performance in both static and ventilated cages. These studies used outbred (ICR), inbred (various), and numerous transgenic strains.

Gaskill and Pritchett-Corning

In the Gaskill and Pritchett-Corning (16) study, the effects of varied cage size on reproduction and behavior were investigated using a very fertile outbred mouse strain (Crl:CD1; Icr) and a standard inbred strain (C57B6NCrl). Mice were housed in nonventilated cages of 4 different sizes: 35, 42.3, 67, and 124 in^2 of floor space. Three breeding scenarios were used: a breeding pair in which the male was removed upon the birth of a litter; a breeding pair in which the male was not removed; and a trio configuration in which the male was not removed. Eight replicate cages of each breeding scenario were tested, for a total of 320 cages evaluated. Behavioral end points recorded by video were activity, grooming, food and water consumption, and aggression. Reproduction was assessed by measuring litter size at birth and weaning, wean weight, time between litters, and pup mortality. The authors concluded that cage size did not significantly affect reproductive or behavioral parameters in any configuration.

Kedl *et al.*

The Kedl (37) study compared the impact of pairwise and trio breeding on litter survival and growth in a panel of 45 inbred and genetically engineered immune models. These included inbred strains, transgenics, and 12 strains with immune phenotypes (autoimmunity, immune deficient, and lymphocyte transgenics), for a total survey of 472 litters generated over a 6-mo period. Litter survival and growth were recorded in static cages providing 75 in^2 of floor space. Pup weight did not differ between the 2 breeding formats. Litter size and survival was significantly increased in trio matings. Inbred, autoimmune, and lymphocyte transgenic strains were statistically indistinguishable from one another in all parameters measured. As expected, variation in some parameters was observed among some strains, but could not be attributed to breeding format.

The authors concluded that "there is parity between trio and pairwise breeding formats for mice across a broad spectrum of strains and genetic alterations." Furthermore, they suggested these results show that space recommendations for a breeding female should not be the same as those for animals that have been weaned. The authors also advocated that their study should be broadly applicable for all investigators rather than requiring independent studies be conducted within each institution.

Yadav *et al.*

In the Yadav *et al.* (38) study, reproductive performance in breeding pairs was compared in a single experiment using

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both static and IVC caging. Reproductive indices (number of pups born, number of pups weaned, number of litters born per pair) were evaluated in 220 pairs of 2 genetically engineered neurologic models, using 110 pairs in each caging type. Pairs were evaluated for the duration of their reproductive cycle. No significant differences were found ($P < 0.05$). The authors concluded that breeding performance was comparable in both cage types and that air quality in static caging could be improved with more frequent cage changing. However, static caging was previously shown to have a negative effect on room air quality (5).

In summary, studies of breeding mice provided evidence that mice are equally productive when configured in pair or trio breeding units. Furthermore, the Whitaker *et al.* (32) and Kedl *et al.* (37) studies independently support a recommendation that, whether in pairs or trios, a breeding female and her litter should be considered a single biologic unit rather than counting individual mice when considering floor space allocation. The eighth Edition of the *Guide* (2011) included a new recommendation that a dam with litter be allocated 51 in^2, without specifying litter size. At up to 13 pups per litter, such as observed in the O'Malley study, each pup would have 3 in^2 of floor space if space for the dam was kept at the recommended 12 in^2. This new recommendation precludes the use of the commonly used trio breeding strategy. Additionally, 2 studies found that reproductive performance and well-being of litters is not improved with culling. Eliminating culling supports alignment with the "3 Rs" [replacement, reduction, refinement (39)], is a better use of all animals and limits unnecessary animal waste. Studies using ventilated caging produced similar conclusions as those that used static caging. These studies, summarized in **Table 2**, encompass evaluations of widely used inbred, transgenic, hybrid, and outbred mouse strains.

IIA. STUDIES USING NONBREEDING MICE: VENTILATED CAGING

Smith *et al.*

In the Smith *et al.* (40, 41) studies, young adult mice of strains B6, BALB/cJ, NOD/LtJ, and FVB/NJ were studied in 3 different IVC cage types (duplex, shoebox, weaning) at 4 densities each (3 of which exceeded *Guide* recommendations) until they were ~3 mo of age. The 4 densities used were the recommended density and up to twice *Guide* recommendations. In duplex cages mice were housed at 4, 6, 8, or 9 mice in each compartment (12.9 in^2/mouse down to 5.7 in^2); in shoebox cages mice were housed at 5, 8, 10, or 12 mice/cage (13.5 in^2 down to 5.6 in^2); in weaning cages mice were housed at 9, 13, 17, or 20 mice/cage (12.5 in^2 down to 5.7 in^2). End points of well-being were survival, weight, food and water consumption, injury, aggressive behavior (fighting), testosterone levels, and cage microenvironment. No impact of increased density in any cage type for either sex of strains B6, BALB/cJ and NOD/LtJ, was found. Males of strain FVB/NJ, however, showed some degree of aggression in all cage types at all densities. This study identified an important strain difference in aggression that occurs regardless of density (42). The health and well-being of female FVB was similar to that of mice of both sexes of other strains and was not affected by cage type or housing density. In another experiment, higher densities were tested using B6 mice housed in weaning cages at 20, 25, 30, or 35 mice/cage [5.6 in^2/mouse down to 3.2 in^2; Smith *et al.* (41, 42)]. No adverse physiologic effects were found at these densities. However, at 3.2 in^2 of floor space ammonia levels in the cage increased above those acceptable in the workplace [OSHA-allowed human standards were used in the absence of standards specific to mice (41)]. Eyes and nasal passages of mice housed at 3.2 in^2/mouse were microscopically normal. In contrast, DiVincenti (33) reported nasal lesions at high ammonia levels.

These studies by Smith and colleagues are some of the most comprehensive in response to the call in the 1996 *Guide* for additional studies. Key advantages of the study design were the use of a large number of animals and the simultaneous use of multiple end points to assess animal well-being. These features were noted as "a great advantage over studies that focus solely on reproduction, medical, physiological, or behavioral parameters" (43) in the summary of a panel discussion on housing density held at an American Association of Laboratory Animal Science meeting before the release of the most current version of the *Guide*.

Nicholson *et al.*

The Nicholson *et al.* (44) study examined 2 inbred strains, C57BL/6J and BALB/cJ, applying an exceptionally wide range of phenotypic measurements, including telemetry. Both sexes of each strain were housed at 4, 6, or 8 mice in a 51.7 in^2 IVC cage (12.9, 8.6, and 6.5 in^2/mouse) from weaning until 5 mo of age using 6 replicate pens per condition. Weight gain, body composition, hematology, serum biochemistry, fecal corticosterone, sex hormones, behavior, home cage telemetry, and cage microenvironment were measured. Although increased density had no effect on most measurements, those measures that did differ were within normal biologic ranges. However, for many measurements only 2 mice/cage were sampled, and the data from sexes and strains were combined for statistical analysis, making it difficult to interpret the significance of these results.

Laber *et al.*

The Laber *et al.* (45) study used the same strains, B6 and BALB, as in Nicholson *et al.* (44) and compared lower and higher densities than those recommended. It differed from the Nicholson study by using a slightly larger cage type and by using only female mice. Using IVC cages with 75 in^2 of floor space, mice were housed at 2, 5, or 10 mice/cage, corresponding to 37.5, 15, and 7.5 in^2 of floor space, respectively, or 0.3, 0.8, and 1.7 times the density the *Guide* recommends. Weight gain, plasma corticosterone, behavior assessed by open field, and immune parameters (T-cell subpopulations) were measured. Data from 5 mice/cage (*Guide* recommended) rarely differed from that of

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10 mice/cage, except for a small but significant reduction in $CD4^+$ T cells found for BALB/c housed at 10 mice/cage compared with 2 and 5 mice/cage. However, values for all densities remained within physiologic normalcy. Most other density-dependent differences were found for BALB only. Effects of density for C57BL/6 mice were significantly different only between mice housed at the lowest (2 mice/cage) and highest (10 mice/cage) densities.

Paigen *et al.*

In Paigen *et al.* (46), housing densities were evaluated using C57BL/6J (B6) females and males housed at 2 densities, 1 slightly above (10.3 in^2/mouse; 5 mice/cage) and 1 about twice the density recommended in the *Guide* (5.7 in^2/mouse; 9 mice/cage). This study differed from previous studies in that observations were made from weaning to 9 mo of age and used a battery of physiologic measurements as well as environmental measures to assess well-being. Measurements included cage microenvironment (ammonia, carbon dioxide, temperature, humidity), food and water consumption, numerous physiologic parameters (hematology, plasma lipids, body weight and composition, electrocardiogram, terminal adrenal weight), fighting, barbering, and hair loss. No differences were found between the 2 density groups housed in the same size IVC cage, suggesting that B6 mice may be housed at higher density without adverse effects on well-being. Some data confirmed previous studies showing decreased heart rate (17, 44) and reduced adrenal weight (47) in the highest density group, suggesting potentially beneficial effects of housing mice at up to twice current recommendations.

Morgan *et al.*

The Morgan *et al.* (48) study tested whether conclusions from Paigen (46) extended to other commonly used inbred strains. Using both sexes of 5 inbred strains housed in duplex and shoebox IVC cages (129S1/SvImJ, A/J, BALB/cBy, B6, DBA/2J), mice were housed at densities of 1, 2, 2.6, and 3 times *Guide* recommendations over 2 study durations, 3 and 8 mo. All measurements used in the 2012 study by Paigen and colleagues were included, as well as additional tests for behavior to measure anxiety (light/dark, open field). Cage type (duplex or shoebox) did not affect any outcomes. As expected, significant strain differences were found for many parameters, but only a few differences were found attributable to density. At higher densities mice had lower heart rate and lower kidney and adrenal weights; these values were within normal physiologic ranges. Observations of reduced heart rate and adrenal weight at higher densities were again consistent with results from earlier studies (17, 44, 46, 47), which may indicate reduced stress at higher densities of mice.

Paigen *et al.*

In Paigen *et al.* (49), a commonly used hybrid strain (129S1/SvImJ × C57BL/6J) was evaluated for effects of housing density using the same end points measured in the Morgan study with added evaluation of immune function. In this study mice were housed in IVC shoebox cages at 4 different densities, from half to twice that recommended by the *Guide*. No density-dependent differences were found, suggesting that the hybrid vigor found in these F1 hybrid mice does not affect its response to increased housing density.

Together, these studies using nonbreeding mice in ventilated cages share conclusions that housing B6, other commonly used inbred strains, and a hybrid strain at densities exceeding current *Guide*-recommended densities had no adverse effects on physiology and behavior and showed some evidence of reduced stress.

IIB. STUDIES USING NONBREEDING MICE: STATIC CAGING

Van Loo *et al.*

In one of the first comprehensive studies of the effects of group size on aggression, Van Loo *et al.* (17) considered both cage size and group size in their evaluation of male BALB/cAnCrlBr mice in non-IVC cages. This study was not a density study but rather a group size study, because investigators kept the number of mice per floor space constant by adjusting the size of the cage. Groups of 3, 5, and 8 mice/cage were used. Aggression was measured by analyzing behaviors for 30-min after cage changing. Investigators found that aggression increased with cage size. The floor space allocated to mice in this study was either the *Guide* recommendation (12.4 in^2/mouse) or less dense (19.4 in^2). With more space than recommended, BALB/c male mice were more likely to fight.

Horn *et al.*

The Horn *et al.* (50) study used C57BL/6NHsd mice in static caging providing slightly less than recommended floor space per mouse and kept floor space the same as mice grew, instead of increasing space as mouse weight increases, as the *Guide* recommends. Because of the large cage size used, the 5.5 in^2/mouse was achieved by housing 25 mice/cage. Body weight, aggression and social behavior, food consumption, cage microenvironment, and morbidity and mortality were measured. The 2 treatment groups were defined by the use of different sanitation practices: in 1 group, bedding and cages were changed weekly; in the other group, bedding was changed weekly and cages changed monthly. No significant differences were found between groups for any of the indices measured. Importantly, this study demonstrated that 25 mice housed together did not experience compromised welfare and floor space per mouse did not need to be increased as mice grew. Although undetermined, one possibility for the lack of fighting among 25 mice is that fighting for dominance occurs only when the number of mice is relatively small and there is more space to fight over.

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TABLE 2. *Summary of studies using breeding mice in static and ventilated cage systems*

Reference	Year	Study focus	Cage ventilation	Mouse strain	Mouse details	Cage floor space		End points	Results
						in^2	cm^2		
O'Malley *et al.* (31)	2008	Effects of culling on reproduction	IVC	ICR	28 pregnant females; first and second generation pups	65 per dam with litter	419	Fecal corticosterone, growth, weaning weight, reproductive behavior	Growth rate slightly reduced with culling; no difference in corticosterone or reproduction between culled and nonculled groups.
Whitaker *et al.* (32)	2009	Effects of cage size on reproduction	IVC	C57BL/6Tac	190 trio breeding units	82, 124	529, 800	# pups born; # pups weaned; time between litters	Larger cages (lower density) were associated with lower pup weight.
DiVincenti *et al.* (33)	2012	Cage microenvironment	IVC	Swiss Webster	Pair and trio breeding units (15 each); litters culled to 6 (pairs) or 12 (trios)	75	484	Cage NH_3, CO_2, humidity, temperature; nasal pathology	Temperature increased in trio cages *vs.* pair cages; no difference in NH_3 or relative humidity; CO_2 higher in trio cages; nasal lesion results inconclusive
Paigen *et al.* (35)	2014	Effect of culling on health of mouse litters	IVC	(C57BL/6 × 129S1/SvImJ) F1	78 trio matings; 468 litters; 3 cull groups: no cull, cull to 4 or 6 pups/litter; weanlings evaluated at 3 mo of age; effect of culling and reproduction evaluated in breeders	78	503	Pups: growth, mortality, hematology, EKG, body composition, organ weights. Breeders: litter size, fecal corticosterone, organ weights	No significant differences among cull groups for pups or breeders.
Braden *et al.* (36)	2017	Effects of breeding configuration on maternal and weanling behavior	IVC	C57BL/6J, 129S6/SvEvTac	98 breeding animals of each strain; pair, trio, and harem configurations used; shoebox cages	67	432	Nest score, pup retrieval, open field, elevated plus maze, tail suspension test, anatomic and clinical pathology, fecal corticosterone, cage ammonia	Trio and harem breeding produced higher wean weights in both strains; B6 produced fewer pups in harem breeding; no negative effects on behavior or pathology in any configuration. Ammonia levels exceeded recommendations in harem breedings.

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TABLE 2. (*continued*)

Reference	Year	Study focus	Cage ventilation	Mouse strain	Mouse details	Cage floor space		End points	Results
						in^2	cm^2		
Kedl *et al.* (37)	2014	Comparison of reproductive performance between pair and trio breeding strategies	Static	46 strains with focus on immunity: C57BL/6J, BALB/cJ, NOD/ShiLtJ, (C57BL/6J xA/J)F1; 12 immune models; 30 transgenic strains	197 pair breeding units; 275 trio breeding units; 472 litters	75	484	Pup weight, litter size, pup survival	No difference in pup weight between breeding strategies; litter size and survival significantly increased in trio matings.
Gaskill and Pritchett-Corning (16)	2015	Effect of cage space on behavior and reproduction	Static	ICR, C57BL/6NCrl	320 cages; 4 cage sizes; 3 breeding configurations. Two study arms, 1 for behavior and 1 for reproduction	35, 42, 67, 124	226, 271, 432, 800	Reproduction: litter size, wean weight; Behavior: video analysis	No differences in reproduction; no behavioral differences in C57BL/6NCrl; some behavioral differences in ICR but did not trend with cage size or configuration.
Yadav *et al.* (38)	2017	Comparison of pair breeding in static and IVC caging	Static *vs.* IVC	Engineered neurologic models: B6C3-Tg (APPswe, PSEN1dE9) 85Dbo/Mmjax; GAP-43 knockout	110 pairs of each strain used in each cage type	IVC: 103; Static: 85.7	IVC: 665; Static: 553	Per dam: no. litters born, no. pups born, no. pups weaned	No significant differences in breeding performance indices between cage types for either strain ($P < 0.05$)

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TABLE 3. *Summary of density studies using nonbreeding adult mice in static and ventilated cage systems*

Reference	Year	Study focus	Cage ventilation	Mouse strain	Floor space/mouse: Mouse details	Floor space/mouse: Cage size, in^2 (mice/cage)	Cage size, cm^2 (mice/cage)	Fold-density *vs.* *Guide*	End points	Density-dependent differences	Trend of difference ($P < 0.01$)	Interpretation
Smith *et al.* (40, 41)	2004, 2005	Density and cage floor space	IVC	C57BL/6J, BALB/cJ, NOD/LtJ, FVB/NJ	3 densities; 3 cage types; 2-mo study; both sexes	~12.5 (4, 5, 9); ~8.6 (6, 8, 13); ~6.6 (8, 10, 17); ~5.6 (9, 12, 20)	81 (4, 5, 9); 55 (6, 8, 13); 43 (8, 10, 17); 36 (9, 12, 20)	1.0×; 1.5×; 1.9×	Weight, food and water consumption, injury, aggressive behavior, survival, testosterone, cage microenvironment	Fighting in FVB/NJ males	Greater aggression in all densities and cage types for FVB/NJ males	All but FVB/NJ males can be housed near twice the density specified in the *Guide*
Smith *et al.* (40)	2004	Density and cage floor space	IVC	C57BL/6J	Weaning cage; 4 densities; 4-wk study; both sexes	5.6 (20); 4.5 (25); 3.8 (30); 3.2 (35)	36 (20); 29 (25); 25 (30); 21 (35)	2.2×; 2.8×; 3.3×; 3.9×	Same as above; add nasal and eye histology	Ammonia concentrations	Ammonia concentrations exceeded acceptable levels for the 2 highest densities (3.8 and 3.2 in^2/mouse)	B6 mice in weaning cages can be housed at up to 2.8 times the density specified in the *Guide*
Laber *et al.* (45)	2008	Density and cage floor space	IVC	C57BL/6NCrl, BALB/cAnNCrl	3 densities; 70-d study; 3 time points; females only	37.5 (2); 15.0 (5); 7.5 (10)	242 (2); 97 (5); 48 (10)	0.3×; 0.8×; 1.7×	Weight gain, corticosterone, behavior, immune parameters	Weight gain	Lower at highest density (BALB)	Increased core temperature in mice at higher densities may reduce metabolic demand for food, resulting in lower consumption and less weight gain[1]
										Plasma corticosterone	Higher at highest density (BALB), but Whitaker *et al.* suggest both strains higher at highest	
										Immune parameters	CD4 + T Cells lower in highest density (BALB)	
Nicholson *et al.* (44)	2009	Increased housing density	IVC	C57BL/6J, BALB/cJ	3 densities; 4-mo study; both sexes	12.9 (4); 8.6 (6); 6.5 (8)	83 (4); 55 (6); 42 (8)	1.0×; 1.5×; 1.9×	Growth, body composition, hematology, hormones, metabolites, telemetry, behavior, fecal corticosterone, adrenal glands, cage microenvironment	Weight gain	Lower at highest density (B6m; BALB f, m)	Increased cage temperature in mice at higher densities may reduce metabolic demand for food, resulting in lower consumption and less weight gain
										Heart rate	Lower at highest density (BALB only)	May indicate reduced stress
										Exploratory behavior	Lower at highest density	Uncertain
										Rearing	Lower at highest density	Uncertain

(*continued on next page*)

TABLE 3. (*continued*)

Reference	Year	Study focus	Cage ventilation	Mouse strain	Floor space/mouse: Mouse details	Floor space/mouse: Cage size, in^2 (mice/cage)	Cage size, cm^2 (mice/cage)	Fold-density *vs.* *Guide*	End points	Density-dependent differences	Trend of difference ($P < 0.01$)	Interpretation
										Fighting	Higher at lowest density (BALB males)	Higher density may resolve aggression in BALB
										Self-grooming	Higher at lowest density	Larger groups may groom each other more
										Relative humidity	Higher at lowest density	Unexpected
										Whisker picking	Higher at highest density (B6)	Typical B6 behavior
										Temperature	Higher at highest density	Expected
										$C0_2$	Higher at highest density	Expected
Paigen *et al.* (46)	2012	Increased housing density	IVC	C57BL/6J	2 densities; 9-mo study; both sexes				Growth, blood chemistries, body composition, EKG, aggression, barbering, mortality, food and water consumption, adrenal weight	Hematology: CHCM	Higher at higher density	Within physiologic normalcy; acceptable
										Cage temperature	Higher at higher density	Expected
										EKG: heart rate	Higher at higher density	Expected
										Cage ammonia	Higher at higher density	Expected; did not exceed acceptable thresholds
										Food consumption	Lower at higher density	Indicates reduced chronic stress
										Body weight	Lower at higher density	Expected
										Adrenal weight	Lower at higher density	Expected
											Lower at higher density	Indicates reduced chronic stress
Morgan *et al.* (48)	2014	Increased housing density in 5 strains	IVC	129S1/SvImJ, A/J, C57BL/6J, BALB/cByJ, DBA/2J	4 densities; 2 cage types; 3- and 8-mo studies	12.9 (4, 6); ~7.6 (7, 10); ~5.6 (9, 14); ~4.8 (11, 16)	83 (4, 6); 49 (7, 10); 36 (9, 14); 31 (11, 16)	2×; 2.6×; 3×	Growth, blood chemistries, body composition, EKG, blood pressure, aggression, barbering, mortality, organ weights	% Body fat	Higher at higher density (129, A, BALB)	Indicates better metabolic health
										Kidney weight	Lower at higher density	Indicates reduced chronic stress
										Heart weight	Lower at higher density	Indicates reduced chronic stress
										Adrenal weight	Lower at higher density	Indicates reduced chronic stress
Paigen *et al.* (49)	2016	Increased density in a hybrid strain	IVC	(C57BL/6J × 129SvImJ)F1	4 densities; shoebox cages; 20-mo study; both sexes	26 (3); 15.6 (5); 9.8 (8); 6.5 (12)	168 (3); 101 (5); 63 (8); 42 (12)	0.5×; 0.8×; 1.2×; 1.8×	Growth, blood pressure, body composition, plasma chemistries, organ weights, flow cytometry (spleen)	None	N/A	B6129F1 mice can be housed at near twice the density specified in the *Guide* with no adverse effects on well-being
Van Loo *et al.* (17)	2001	Influence of group and cage size on male aggression	Static	BALB/cAnNCrlBr	2 densities; 3 group sizes; 2.5-mo study; males	12.4 (3, 5, 8); 19.4 (3, 5, 8)	80 (3, 5, 8); 125 (3, 5, 8)	1.0×; 0.62×	Behavior, food and water consumption, urinary corticosterone, testosterone, organ weights	Agonistic behavior	Higher in larger cages, regardless of density	Male mice fight more with more floor space

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TABLE 3. (*continued*)

Reference	Year	Study focus	Cage ventilation	Mouse strain	Floor space/mouse		Cage size, cm^2 (mice/cage)	Fold-density *vs.* *Guide*	End points	Density-dependent differences	Trend of difference ($P < 0.01$)	Interpretation
					Mouse details	Cage size, in^2 (mice/cage)						
Horn *et al.* (50)	2012	Increased housing density; no stratification for mouse weight	Static	C57BL/6NHsd	<10 to >25 g mice housed at 11 density; wean cage; bedding (3 types) change weekly; clean cages weekly or monthly; 2-mo study; both sexes	5.5 (25)	35 (25)	1.0×; 1.5×; 2.2×; 2.7×	Body weight, behavior, food consumption, fecal corticosterone, cage microenvironment, airway histology, mortality	None	N/A	For C57BL/6NHsd, as many as 25 mice can be housed together with no adverse effects on well-being
Bailoo *et al.* (51)	2018	Group size and cage type	Static	C57BL/6ByJ, BALB/cByJ	3 densities; 3 cage types; 13-wk study; both sexes	7.1 (8); 11.5 (5); 17.5 (3, 8); 25.4 (5); 44.5 (3, 8); 74.4 (5); 124 (3)	46 (8); 74 (5); ~113 (3, 8); 164 (5); ~287 (3, 8); 480 (5); 800 (3)	1.7×; 1.0×; 0.7×; 0.5×; 0.3×; 0.2×; 0.1×	Growth, food and water intake, fecal glucocorticoid metabolites, open field test, guessing task, home cage behavior	Food and water intake	Higher at lowest density	May be due to increased need for metabolic stability

[1]Toth *et al.* (64).

Bailoo *et al.*

Bailoo *et al.* (51) conducted a large study in Europe that compared the effects of both group size and floor space allowance using both sexes of C57BL/6ByJ and BALB/cByJ mice. Three cage sizes were used (57.4, 127.1, 372 in^2); the 2 smaller cages are comparable in size to United States duplex and weaning cages, respectively. The third cage type, predominantly used for housing pet mice, was ~3 times as large as a weaning cage. Mice were housed in groups of 3, 5, or 8 in each cage type. Using 6 replicate cages per condition, a total of 1152 mice in 216 cages were evaluated. The experimental design produced one condition in which floor space per mouse (11.5 in^2) approximated the current *Guide* recommendation and one condition in which floor space per mouse was half the current recommendation (7.1 in^2). The remaining cages provided more space per mouse than *Guide* recommendations, ranging from 17 to 124 in^2/mouse. No significant differences were found for fecal glucocorticoid metabolites, open field behavior, brain function (measured by a guessing task), or home cage behavior. Groups of 3 mice/cage generally ate and drank more than larger groups regardless of cage type, an observation that has been made in other studies (44–46). For male BALB/c mice, aggression increased with increasing group size regardless of cage type, as previously reported for this strain (17, 44). The authors conclude that space allowance has little impact on well-being in laboratory mice and that space allowance may not be the most important aspect of housing conditions that affects well-being.

Results from these studies using static caging to evaluate effects of housing density or group size were consistent with those from studies using ventilated cages. The conclusions from studies using nonbreeding mice are summarized in **Table 3**. Collectively, these investigations using nonbreeding mice include 7 inbred strains and 1 hybrid strain. Across this range of commonly used strains, there was broad agreement that well-being is maintained with increased housing density in both ventilated and static caging systems. However, a few strains, such as FVB and BALB/c, are particularly aggressive regardless of density (40). Therefore, adjustments to housing configurations will be required when using aggressive strains, rather than trying to apply a uniform standard to all.

GAPS IN MOUSE DENSITY RESEARCH

A surge in density studies in the last 2 decades, with widely varying designs, has contributed greatly to our understanding of the effects of housing conditions on well-being of research mice. Many of these studies have added to the evidence that, for many strains, adding more mice to a cage is not detrimental to their well-being. Some studies even indicate that well-being may be improved at densities exceeding *Guide* recommendations, as shown by reductions in heart rate, adrenal weight, and aggression. Nevertheless, these important gaps in our knowledge remain:

- *Low density housing*: What is the impact on well-being when mice are housed individually? Does

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having 2 mice improve effects of social isolation? How does paired housing compare with 3 mice/cage? Additional studies that include 1, 2, and 3 mice/cage, in addition to higher densities, would be useful.

- *Behavioral assessments*: More sophisticated methods for testing complex animal behaviors have recently emerged, many of which focus on home cage behavior. Using a broader array of these tests within a single experiment will allow better understanding of what aspects of behavior are affected by housing density. Here, a cautionary note is in order about interpreting whether behavioral data is beneficial or detrimental in the context of the research setting.
- *Dietary effects*: Recent studies have suggested that high fat diets may reduce chronic social stress (52, 53) and may warrant further investigation of the impact of diet on well-being in density studies.
- *Static caging*: This caging strategy is still widely used. More studies at low and high density are needed using non-IVC housing. There has been considerable data to support increased density in IVC systems, but increasing density in static cages is likely to increase allergen exposure to humans, and this should be taken into account. Static caging is widely used, due to tradition or cost of moving to ventilated caging. However, cost recovery from such an investment may be realized by a reduction in frequency of cage changing, reducing disruption of mice, and improving the health of animal handlers (4, 5).
- *Cage microenvironment*: Standards for ammonia and carbon dioxide levels within mouse cages have not been determined. Currently, comparisons are made to OSHA standards set for human exposures. It would be useful to determine acceptable thresholds for mice.
- *Hybrid and outbred strains*: With emerging goals for precision medicine in biomedical research, hybrid and outbred mouse populations are increasingly being used (54). Only a few studies to date have investigated effects of housing density in these resources.
- *Technical replicates*: Studies in this review still vary widely in design, mostly in the number of replicate cages used. Results are likely to be most reproducible and more easily interpretable if adequate technical replicates are included. Power calculations should be performed to estimate the number of replicate cages and mice to use, taking into account the necessity to correct statistical significance for multiple testing when many parameters are measured. This calculation varies depending on the number and variance of the parameters measured.

ENRICHMENT

Cage environment other than air quality is a confounding issue when assessing animal well-being. The use of enrichment has varied effects on many behavioral parameters and social interactions (55–58) and is a complex treatment that is beyond the scope of this review. Enrichment strategies for laboratory rodents was the focus of a special issue of the *Institute for Laboratory Animal Research Journal* in 2005 (59). Baumans and Van Loo have contributed significantly to this field with studies of how environmental refinement influences the well-being of mice and other common laboratory species (60–62). Many institutes now require enrichment for singly housed animals. In the breeding study by Whitaker *et al.* (32), more mice were weaned and their 21-d weights were increased when enrichment (Nestlet, plastic tunnel, nylon ring, running wheel) was added to breeding cages, regardless of cage size. O'Malley *et al.* (31) used 2 different bedding materials (wire grid or wood chips with cotton nesting material) in their study of the effects of culling on reproduction and found no effect of bedding on any of the parameters measured. Suggestions for effective use of enrichment that may improve well-being of laboratory mice are provided in a review by Smith and Corrow (42). More recently, André and colleagues measured effects of simple enrichment materials on 164 physiologic parameters in 2 strains and found improvement in animal welfare without impairing experimental outcome (63).

DISCUSSION

The studies summarized in this review address the effects of housing density in both breeding and nonbreeding circumstances, using a variety of physiologic and behavioral measures. They agree on 3 major points: *1*) culling of litters does not improve the well-being of surviving pups; *2*) trio breeding configurations are not detrimental to the well-being of adult mice or their preweaned litters; and *3*) nonbreeding research mice housed at densities exceeding those recommended in the *Guide* demonstrate that for most strains a significant decrease in floor space allowance does not negatively impact baseline physiology and well-being and can reduce stress.

This review adds to the important review by Whittaker *et al.* (3) and agrees with several of its conclusions, namely that "scientific evidence suggests that space *per se* has little effect on reproductive parameters and that increasing individual space allowance may actually increase aggressive encounters," and that reduced space may have little impact on well-being. Although the Whittaker review mentioned that it is a challenge to interpret data because of few studies and varied experimental design, since that review there have been at least 11 robust studies published addressing housing density that can be compared. In the current review, we have separated studies by whether they used ventilated or nonventilated caging systems and by whether studies were carried out on breeding mice or on experimental adult mice. The Whittaker review also concluded that increased density leads to increased stress and lower immunity *via* the hypothalamic-pituitary-adrenal axis, but this was based on a small number of older studies that differ from more recent studies included in this review. Finally, Whittaker *et al.* recommend that additional studies should keep group size constant and use varied cage sizes. This recommendation will be difficult to meet because many institutions have made a commitment to cage sizes and it is far easier to change the number of mice.

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In summarizing a panel discussion evaluating the rationale for decreasing floor space held at 2006 American Association for Laboratory Animal Science National Meeting, the authors noted that the issues of housing density are complex and that there were not enough new studies at that time to warrant changing recommendations in the next version of the *Guide* (43). Additionally, they noted that "reassessment and updating of the *Guide's* space recommendations is certainly overdue." The numerous studies summarized in this review have since addressed floor space allocation, providing a substantial baseline of new information for evaluating appropriate policies. Moreover, because the many studies that have been carried out in the intervening years are consistent in their findings, this should eliminate any requirement to repeat such studies within each institution, thereby reducing the overuse of mice in research. FJ

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AUTHOR CONTRIBUTIONS

Both authors designed the strategy for presenting previous work, analyzed the outcomes of previous studies, provided intellectual contributions to discussion, and wrote the manuscript.

REFERENCES

1. National Research Council (US). (2011) *Guide for the Care and Use of Laboratory Animals*, 8th ed., National Academies Press (US), Washington, DC, USA
2. Institute of Laboratory Animal Resources (US). (1996) *Guide for the Care and Use of Laboratory Animals*, National Academies Press (US), Washington, DC
3. Whittaker, A. L., Howarth, G. S., and Hickman, D. L. (2012) Effects of space allocation and housing density on measures of wellbeing in laboratory mice: a review. *Lab. Anim.* **46**, 3–13
4. Smith, E., Stockwell, J. D., Schweitzer, I., Langley, S. H., and Smith, A. L. (2004) Evaluation of cage micro-environment of mice housed on various types of bedding materials. *Contemp. Top. Lab. Anim. Sci.* **43**, 12–17
5. Schweitzer, I. B., Smith, E., Harrison, D. J., Myers, D. D., Eggleston, P. A., Stockwell, J. D., Paigen, B., and Smith, A. L. (2003) Reducing exposure to laboratory animal allergens. *Comp. Med.* **53**, 487–492
6. Reeb-Whitaker, C. K., Paigen, B., Beamer, W. G., Bronson, R. T., Churchill, G. A., Schweitzer, I. B., and Myers, D. D. (2001) The impact of reduced frequency of cage changes on the health of mice housed in ventilated cages. *Lab. Anim.* **35**, 58–73
7. Champy, M. F., Selloum, M., Piard, L., Zeitler, V., Caradec, C., Chambon, P., and Auwerx, J. (2004) Mouse functional genomics requires standardization of mouse handling and housing conditions. *Mamm. Genome* **15**, 768–783
8. Lloyd, K. C., Meehan, T., Beaudet, A., Murray, S., Svenson, K., McKerlie, C., West, D., Morse, I., Parkinson, H., Brown, S., Mallon, A. M., and Moore, M. (2015) Precision medicine: look to the mice. *Science* **349**, 390
9. De Angelis, M. H., Nicholson, G., Selloum, M., White, J., Morgan, H., Ramirez-Solis, R., Sorg, T., Wells, S., Fuchs, H., Fray, M., Adams, D. J., Adams, N. C., Adler, T., Aguilar-Pimentel, A., Ali-Hadji, D., Amann, G., André, P., Atkins, S., Auburtin, A., Ayadi, A., Becker, J., Becker, L., Bedu, E., Bekeredjian, R., Birling, M. C., Blake, A., Bottomley, J., Bowl, M., Brault, V., Busch, D. H., Bussell, J. N., Calzada-Wack, J., Cater, H., Champy, M. F., Charles, P., Chevalier, C., Chiani, F., Codner, G. F., Combe, R., Cox, R., Dalloneau, E., Dierich, A., Di Fenza, A., Doe, B., Duchon, A., Eickelberg, O., Esapa, C. T., El Fertak, L., Feigel, T., Emelyanova, I., Estabel, J., Favor, J., Flenniken, A., Gambadoro, A., Garrett, L., Gates, H., Gerdin, A. K., Gkoutos, G., Greenaway, S., Glasl, L., Goetz, P., Da Cruz, I. G., Götz, A., Graw, J., Guimond, A., Hans, W., Hicks, G., Hölter, S. M., Höfler, H., Hancock, J. M., Hoehndorf, R., Hough, T., Houghton, R., Hurt, A., Ivandic, B., Jacobs, H., Jacquot, S., Jones, N., Karp, N. A., Katus, H. A., Kitchen, S., Klein-Rodewald, T., Klingenspor, M., Klopstock, T., Lalanne, V., Leblanc, S., Lengger, C., le Marchand, E., Ludwig, T., Lux, A., McKerlie, C., Maier, H., Mandel, J. L., Marschall, S., Mark, M., Melvin, D. G., Meziane, H., Micklich, K., Mittelhauser, C., Monassier, L., Moulaert, D., Muller, S., Naton, B., Neff, F., Nolan, P. M., Nutter, L. M., Ollert, M., Pavlovic, G., Pellegata, N. S., Peter, E., Petit-Demoulière, B., Pickard, A., Podrini, C., Potter, P., Pouilly, L., Puk, O., Richardson, D., Rousseau, S., Quintanilla-Fend, L., Quwailid, M. M., Racz, I., Rathkolb, B., Riet, F., Rossant, J., Roux, M., Rozman, J., Ryder, E., Salisbury, J., Santos, L., Schäble, K. H., Schiller, E., Schrewe, A., Schulz, H., Steinkamp, R., Simon, M., Stewart, M., Stöger, C., Stöger, T., Sun, M., Sunter, D., Teboul, L., Tilly, I., Tocchini-Valentini, G. P., Tost, M., Treise, I., Vasseur, L., Velot, E., Vogt-Weisenhorn, D., Wagner, C., Walling, A., Weber, B., Wendling, O., Westerberg, H., Willershäuser, M., Wolf, E., Wolter, A., Wood, J., Wurst, W., Yildirim, A. O., Zeh, R., Zimmer, A., Zimprich, A., Holmes, C., Steel, K. P., Herault, Y., Gailus-Durner, V., Mallon, A. M., and Brown, S. D; EUMODIC Consortium. (2015) Analysis of mammalian gene function through broad-based phenotypic screens across a consortium of mouse clinics. *Nat. Genet.* **47**, 969–978
10. Fuchs, H., Aguilar-Pimentel, J. A., Amarie, O. V., Becker, L., Calzada-Wack, J., Cho, Y. L., Garrett, L., Holter, S. M., Irmler, M., Kistler, M., Kraiger, M., Mayer-Kuckuk, P., Moreth, K., Rathkolb, B., Rozman, J., da Silva Buttkus, P., Treise, I., Zimprich, A., Gampe, K., Hutterer, C., Stoger, C., Leuchtenberger, S., Maier, H., Miller, M., Scheideler, A., Wu, M., Beckers, J., Bekeredjian, R., Brielmeier, M., Busch, D. H., Klingenspor, M., Klopstock, T., Ollert, M., Schmidt-Weber, C., Stoger, T., Wolf, E., Wurst, W., Yildirim, A. O., Zimmer, A., Gailus-Durner, V., and Hrabe de Angelis, M. (2018) Understanding gene functions and disease mechanisms: phenotypingp pipelines in the German Mouse Clinic. *Behav. Brain Res.* **352**, 187–196
11. Laughlin, M. R., Lloyd, K. C., Cline, G. W., and Wasserman, D. H.; Mouse Metabolic Phenotyping Centers Consortium. (2012) NIH mouse metabolic phenotyping centersmmpc: the power of centralized phenotyping. *Mamm. Genome* **23**, 623–631
12. Svenson KL, Bogue MA, and Peters LL. (2003) Invited review: identifyingi new mouse models of cardiovascular disease: a review of high-throughput screens of mutagenized and inbred strains. *J. Appl. Physiol. (1985)* **94**, 1650—1659; discussion 1673
13. Zheng, Q. Y., Johnson, K. R., and Erway, L. C. (1999) Assessment of hearing in 80 inbred strains of mice by ABR threshold analyses. *Hear. Res.* **130**, 94–107
14. Brown, R. E., and Wong, A. A. (2007) The influence of visual ability on learning and memory performance in 13 strains of mice. *Learn. Mem.* **14**, 134–144
15. Wohleb, E. S., Powell, N. D., Godbout, J. P., and Sheridan, J. F. (2013) Stress-induced recruitment of bone marrow-derived monocytes to the brain promotes anxiety-like behavior. *J. Neurosci.* **33**, 13820–13833
16. Gaskill, B. N., and Pritchett-Corning, K. R. (2015) The effect of cage space on behavior and reproduction in Crl:CD1(Icr) and C57BL/6NCrl laboratory mice. *PLoS One* **10**, e0127875
17. Van Loo, P. L., Mol, J. A., Koolhaas, J. M., Van Zutphen, B. F., and Baumans, V. (2001) Modulation of aggression in male mice: influence of group size and cage size. *Physiol. Behav.* **72**, 675–683
18. Jhuang, H., Garrote, E., Mutch, J., Yu, X., Khilnani, V., Poggio, T., Steele, A. D., and Serre, T. (2010) Automated home-cage behavioural phenotyping of mice. *Nat. Commun.* **1**, erratum: 3
19. Kabra, M., Robie, A. A., Rivera-Alba, M., Branson, S., and Branson, K. (2013) JAABA: interactive machine learning for automatic annotation of animal behavior. *Nat. Methods* **10**, 64–67
20. Weber, E. M., Dallaire, J. A., Gaskill, B. N., Pritchett-Corning, K. R., and Garner, J. P. (2017) Aggression in group-housed laboratory mice: why can't we solve the problem? *Lab Anim. (NY)* **46**, 157–161
21. Cryan, J. F., Mombereau, C., and Vassout, A. (2005) The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. *Neurosci. Biobehav. Rev.* **29**, 571–625
22. Bains, R. S., Wells, S., Sillito, R. R., Armstrong, J. D., Cater, H. L., Banks, G., and Nolan, P. M. (20188) Assessing mouse behaviour throughout

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the light/dark cycle using automated in-cage analysis tools. *J. Neurosci. Methods* **300**, 37–47
23. Patel, T. P., Gullotti, D. M., Hernandez, P., O'Brien, W. T., Capehart, B. P., Morrison III, B., Bass, C., Eberwine, J. E., Abel, T., and Meaney, D. F. (2014) An open-source toolbox for automated phenotyping of mice in behavioral tasks. *Front. Behav. Neurosci.* **8**, 349
24. Salem, G. H., Dennis, J. U., Krynitsky, J., Garmendia-Cedillos, M., Swaroop, K., Malley, J. D., Pajevic, S., Abuhatzira, L., Bustin, M., Gillet, J. P., Gottesman, M. M., Mitchell, J. B., and Pohida, T. J. (2015) SCORHE: a novel and practical approach to video monitoring of laboratory mice housed in vivarium cage racks. *Behav. Res. Methods* **47**, 235–250
25. Sá-Rocha, V. M., Sá-Rocha, L. C., and Palermo-Neto, J. (2006) Variations in behavior, innate immunity and host resistance to B16F10 melanoma growth in mice that present social stable hierarchical ranks. *Physiol. Behav.* **88**, 108–115
26. Schmidt, M. V., Scharf, S. H., Liebl, C., Harbich, D., Mayer, B., Holsboer, F., and Müller, M. B. (2010) A novel chronic social stress paradigm in female mice. *Horm. Behav.* **57**, 415–420
27. Sommershof, A., Basler, M., Riether, C., Engler, H., and Groettrup, M. (2011) Attenuation of the cytotoxic T lymphocyte response to lymphocytic choriomeningitis virus in mice subjected to chronic social stress. *Brain Behav. Immun.* **25**, 340–348
28. Young, E. E., Vichaya, E. G., Reusser, N. M., Cook, J. L., Steelman, A. J., Welsh, C. J., and Meagher, M. W. (2013) Chronic social stress impairs virus specific adaptive immunity during acute Theiler's virus infection. *J. Neuroimmunol.* **254**, 19–27
29. Levin, S., Šemler, D., and Ruben, Z. (1993) Effects of two weeks of feed restriction on some common toxicologic parameters in Sprague-Dawley rats. *Toxicol. Pathol.* **21**, 1–14
30. Pérez, C., Canal, J. R., Domínguez, E., Campillo, J. E., Guillén, M., and Torres, M. D. (1997) Individual housing influences certain biochemical parameters in the rat. *Lab. Anim.* **31**, 357–361
31. O'Malley, J., Dambrosia, J. M., and Davis, J. A. (2008) Effect of housing density on reproductive parameters and corticosterone levels in nursing mice. *J. Am. Assoc. Lab. Anim. Sci.* **47**, 9–15
32. Whitaker, J., Moy, S. S., Godfrey, V., Nielsen, J., Bellinger, D., and Bradfield, J. (2009) Effects of cage size and enrichment on reproductive performance and behavior in C57BL/6Tac mice. *Lab Anim. (NY)* **38**, 24–34
33. DiVincenti, L., Jr., Moorman-White, D., Bavlov, N., Garner, M., and Wyatt, J. (2012) Effects of housing density on nasal pathology of breeding mice housed in individually ventilated cages. *Lab Anim. (NY)* **41**, 68–76
34. Gordon, C. J. (2012) Thermal physiology of laboratory mice: defining thermoneutrality. *J. Therm. Biol.* **37**, 654–685
35. Paigen, B., Marion, M. A., Stearns, T. M., Harper, J. M., and Svenson, K. L. (2014) The effect of culling on health and physiology of mouse litters. *Lab. Anim.* **48**, 207–215
36. Braden, G. C., Rasmussen, S., Monette, S., and Tolwani, R. J. (2017) Effects of breeding configuration on maternal and weanling behavior in laboratory mice. *J. Am. Assoc. Lab. Anim. Sci.* **56**, 369–376
37. Kedl, R. M., Wysocki, L. J., Janssen, W. J., Born, W. K., Rosenbaum, M. D., Granowski, J., Kench, J. A., Fong, D. L., Switzer, L. A., Cruse, M., Huang, H., Jakubzick, C. V., Kosmider, B., Takeda, K., Stranova, T. J., Klumm, R. C., Delgado, C., Tummala, S., De Langhe, S., Cambier, J., Haskins, K., Lenz, L. L., and Curran-Everett, D. (2014) General parity between trio and pairwise breeding of laboratory mice in static caging. *J. Immunol.* **193**, 4757–4760
38. Yadav, S., Yadav, I., Pratap, K., Tiwari, P. K., and Singh, V. P. (2017) Reproductive performance of genetically engineered mice housed in different housing systems. *Lab. Anim. Res.* **33**, 68–75
39. Russell, W. M. S., and Burch, R. L. (1959) *The Principles of Humane Experimental Technique*, Methuen, London, United Kingdom
40. Smith, A. L., Mabus, S. L., Muir, C., and Woo, Y. (2005) Effects of housing density and cage floor space on three strains of young adult inbred mice. *Comp. Med.* **55**, 368–376
41. Smith, A. L., Mabus, S. L., Stockwell, J. D., and Muir, C. (2004) Effects of housing density and cage floor space on C57BL/6J mice. *Comp. Med.* **54**, 656–663
42. Smith, A. L., and Corrow, D. J. (2005) Modifications to husbandry and housing conditions of laboratory rodents for improved well-being. *ILAR J.* **46**, 140–147
43. Foltz, C., Carbone, L., DeLong, D., Rollin, B. E., Van Loo, P., Whitaker, J., and Wolff, A. (2007) Considerations for determining optimal mouse caging density. *Lab Anim. (NY)* **36**, 40–49
44. Nicholson, A., Malcolm, R. D., Russ, P. L., Cough, K., Touma, C., Palme, R., and Wiles, M. V. (2009) The response of C57BL/6J and BALB/cJ mice to increased housing density. *J. Am. Assoc. Lab. Anim. Sci.* **48**, 740–753
45. Laber, K., Veatch, L. M., Lopez, M. F., Mulligan, J. K., and Lathers, D. M. (2008) Effects of housing density on weight gain, immune function, behavior, and plasma corticosterone concentrations in BALB/c and C57BL/6 mice. *J. Am. Assoc. Lab. Anim. Sci.* **47**, 16–23
46. Paigen, B., Svenson, K. L., Von Smith, R., Marion, M. A., Stearns, T., Peters, L. L., and Smith, A. L. (2012) Physiological effects of housing density on C57BL/6J mice over a 9-month period. *J. Anim. Sci.* **90**, 5182–5192
47. Peters, A., and Festing, M. (1990) Population density and growth rate in laboratory mice. *Lab. Anim.* **24**, 273–279
48. Morgan, J. L., Svenson, K. L., Lake, J. P., Zhang, W., Stearns, T. M., Marion, M. A., Peters, L. L., Paigen, B., and Donahue, L. R. (2014) Effects of housing density in five inbred strains of mice. *PLoS One* **9**, e90012
49. Paigen, B., Currer, J. M., and Svenson, K. L. (2016) Effects of varied housing density on a hybrid mouse strain followed for 20 months. *PLoS One* **11**, e0149647
50. Horn, M. J., Hudson, S. V., Bostrom, L. A., and Cooper, D. M. (2012) Effects of cage density, sanitation frequency, and bedding type on animal wellbeing and health and cage environment in mice and rats. *J. Am. Assoc. Lab. Anim. Sci.* **51**, 781–788
51. Bailoo, J. D., Murphy, E., Varholick, J. A., Novak, J., Palme, R., and Würbel, H. (2018) Evaluation of the effects of space allowance on measures of animal welfare in laboratory mice. *Sci. Rep.* **8**, 713
52. Del Rio, D., Morales, L., Ruiz-Gayo, M., and Del Olmo, N. (2016) Effect of high-fat diets on mood and learning performance in adolescent mice. *Behav. Brain Res.* **311**, 167–172
53. Finger, B. C., Dinan, T. G., and Cryan, J. F. (2011) High-fat diet selectively protects against the effects of chronic social stress in the mouse. *Neuroscience* **192**, 351–360
54. Svenson, K. L., Gatti, D. M., Valdar, W., Welsh, C. E., Cheng, R., Chesler, E. J., Palmer, A. A., McMillan, L., and Churchill, G. A. (2012) High-resolution genetic mapping using the Mouse Diversity outbred population. *Genetics* **190**, 437–447
55. Clipperton-Allen, A. E., Ingrao, J. C., Ruggiero, L., Batista, L., Ovari, J., Hammermueller, J., Armstrong, J. N., Bienzle, D., Choleris, E., and Turner, P. V. (2015) Long-term provision of environmental resources alters behavior but not physiology or neuroanatomy of male and female BALB/c and C57BL/6 mice. *J. Am. Assoc. Lab. Anim. Sci.* **54**, 718–730
56. Hutchinson, E. K., Avery, A. C., and Vandewoude, S. (2012) Environmental enrichment during rearing alters corticosterone levels, thymocyte numbers, and aggression in female BALB/c mice. *J. Am. Assoc. Lab. Anim. Sci.* **51**, 18–24
57. Õkva, K., Nevalainen, T., and Pokk, P. (2013) The effect of cage shelf on the behaviour of male C57BL/6 and BALB/c mice in the elevated plus maze test. *Lab. Anim.* **47**, 220–222
58. Pautassi, R. M., Suárez, A. B., Hoffmann, L. B., Rueda, A. V., Rae, M., Marianno, P., and Camarini, R. (2017) Effects of environmental enrichment upon ethanol-induced conditioned place preference and pre-frontal BDNF levels in adolescent and adult mice. *Sci. Rep.* **7**, 8574
59. Overall, K. L., and Dyer, D. (2005) Enrichment strategies for laboratory animals from the viewpoint of clinical veterinary behavioral medicine: emphasis on cats on dogs. *ILAR J.* **46**, 202–215
60. Baumans, V., and Van Loo, P. L. (2013) How to improve housing conditions of laboratory animals: the possibilities of environmental refinement. *Vet. J.* **195**, 24–32
61. Van de Weerd, H. A., Van Loo, P. L., and Baumans, V. (2004) Environmental enrichment: room for reduction? *Altern. Lab. Anim.* **32**(Suppl 2), 69–71
62. Van Loo, P. L., Blom, H. J., Meijer, M. K., and Baumans, V. (2005) Assessment of the use of two commercially available environmental enrichments by laboratory mice by preference testing. *Lab. Anim.* **39**, 58–67
63. André, V., Gau, C., Scheideler, A., Aguilar-Pimentel, J. A., Amarie, O. V., Becker, L., Garrett, L., Hans, W., Hölter, S. M., Janik, D., Moreth, K., Neff, F., Östereicher, M., Racz, I., Rathkolb, B., Rozman, J., Bekeredjian, R., Graw, J., Klingenspor, M., Klopstock, T., Ollert, M., Schmidt-Weber, C., Wolf, E., Wurst, W., Gailus-Durner, V., Brielmeier, M., Fuchs, H., and Hrabé de Angelis, M. (2018) Laboratory mouse housing conditions can be improved using common environmental enrichment without compromising data. *PLoS Biol.* **16**, e2005019
64. Toth, L. A., Trammell, R. A., and Ilsley-Woods, M. (2015) Interactions between housing density and ambient temperature in the cage environment: effects on mouse physiology and behavior. *J. Am. Assoc. Lab. Anim. Sci.* **54**, 708–717

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